

REMARKS

Prior to the initial examination of the present application, Applicant wishes to have this amendment entered. While adding no new subject matter to the application, Applicant has revised the Specification and the abstract to meet standard U.S. formatting requirements and converted the European style of multiple dependent claims to the U.S. format of single dependency.

Given the above amendments to the claims and the comments herein, Applicant respectfully requests notice of allowance of all pending claims at the earliest convenience. However, should the Examiner envision further rejection or comment, he is invited to telephone the undersigned.

Respectfully submitted,



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Use of N₂O in the treatment of post-ischaemic ischemic
brain cell deterioration

The invention relates to the use of nitrous oxide (N₂O)
5 or of an N₂O donor for producing all or part of a medicinal product intended to treat or prevent post-ischaemic ischemic brain cell deterioration, in particular deterioration subsequent to a stroke, especially all or part of an inhalable gaseous
10 medicinal product, in humans or animals.

In cerebral ischaemia ischemia subsequent to a stroke, and in strokes in general, a functional alteration of many neurotransmission systems is usually noted from a
15 neurochemical point of view, in particular an increase in the release of glutamate, the excitotoxicity and contribution of which to neuronal death are known, as recalled by *Dirnagl et al., Trends Neurosci, 22: 391, 1999.*

20 Moreover, from a functional point of view, in the case of global ischaemia ischemia in the rat, an increase is observed in locomotor activity, in particular described by *Wang and Corbett, Brain Res., 533: 78, 1990; Baldwin et al., Neurodegeneration 2: 139, 1993*, the development of which is generally attributed to an alteration in cognitive functions of spatial recognition rather than to an alteration in sensory-motor functions.

30 As a result, a potential therapeutic role for ionotropic and metabotropic glutameric receptor antagonists have been suspected, in particular by *Chazot, Curr Opin Invest Drugs 1: 370, 2000; Drian et al., Neurochem Int 38: 509, 2001.*

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It also appears that the deleterious effects of known cerebral ~~ischaemias~~ ~~ischemias~~ appear to involve localized ~~ischaemias~~ ischemias which are thought to be caused by glutameric excitotoxicity.

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In fact, the therapeutic potential of glutameric receptor antagonists is often put forward in the treatment of neuropathologies of excitotoxic origin, in particular cerebral ~~ischaemia~~ ischemia, as described by 10 *Dirnagl et al., Trends Neurosci 22: 391, 1999*, and productive disorders, as described by *Benes, Brain Res. Review 31: 251, 2000*.

15 However, the physiology of glutameric receptors is complex and it appears that the high affinity antagonists may also exhibit neurotoxic properties, according to *Burns et al., Psychopharmacology 115: 516, 1994*.

20 Thus, a potential therapeutic advantage of low affinity antagonists, in particular for NMDA, has recently been proposed by *Palmer and Widzowski, Amino acids 19: 151, 2000*.

25 Moreover, document WO-A-02/09731 is also known, which relates to the use of CO, optionally supplemented with another gas, for treating inflammations of the upper respiratory tracts or of the bronchi. That document therefore targets the treatment of pathologies such as 30 asthma, cystic fibrosis, pneumopathies or the like.

In addition, document EP-A-861672 teaches a method of treatment which can be used in emergency situations by administering various gases. However, this does not 35 relate to post-ischaemic ischemic brain cell degradations subsequent to strokes.

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Finally, document FR-A-2812545 teaches a combination of gas and active product intended to treat or prevent pain. The active product is an analgesic, an anti-inflammatory, an anti-pyretic or the like.

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To date, no effective medical product therefore exists for preventing or treating, at least partially, post-ischaemic ischemic brain cell degradation subsequent to strokes.

10

The present invention falls within this context, and aims to provide all or part of a medicinal product which can be used for preventing, decreasing or treating any post-ischaemic ischemic brain cell deterioration, in particular subsequent to a stroke, in humans or animals.

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The invention therefore relates to the use of nitrous oxide (N_2O) or of an N_2O donor for producing all or part of a medicinal product intended to treat, minimize or prevent post-ischaemic ischemic brain cell deterioration.

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Depending on the case, the use of the invention may comprise one or more of the following technical characteristics:

- all or part of the gaseous medicinal product is in inhalable form;
- 25 - the post-ischaemic ischemic brain deterioration results in or is subsequent to a stroke;
- 30 - the nitrous oxide (N_2O) or the nitrous oxide donor is in gaseous form or is included in a gas or a mixture of gases;

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- the medicinal product contains an effective proportion of nitrous oxide (N_2O) or of N_2O donor;
- the medicinal product also contains xenon or a xenon donor, the xenon or the xenon donor being in gaseous form or being included in a gas or a mixture of gases;
- the medicinal product contains an effective proportion of xenon or of a xenon donor;
- the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen;
- the medicinal product contains an amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume;
- the medicinal product contains an amount ranging up to approximately 80% by volume of N_2O or of N_2O donor, preferably up to 75% of N_2O ;
- the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

The invention therefore also relates to an inhalable medicinal product with neuroprotective action in the brain, containing an effective amount of nitrous oxide (N_2O) or of a donor of such a compound, in particular intended to treat, minimize or prevent post-ischaemic ischemic brain cell deterioration.

According to the case, the medicinal product of the invention may comprise one or more of the following technical characteristics:

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- it contains an amount ranging up to 80% by volume of gaseous N₂O;
- it contains an effective amount of nitrous oxide 5 (N₂O) or of a donor of such a compound;
- it contains an effective amount of xenon or of a donor of such a compound;

10 - it contains an amount which is less than 60% by volume of xenon;

- it also contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

15 The idea on which the present invention is based is to take advantage of the NMDA receptor antagonist properties of gaseous N₂O, optionally supplemented with xenon, for their neuroprotective nature, in prevention 20 or treatment of post-ischaemic ischemic pathologies subsequent to strokes.

In fact, recent studies, carried out *in vitro*, have shown that N₂O, and also xenon, can potentially behave 25 like low-affinity antagonists of glutameric receptors for N-methyl-D-aspartate, NMDA (*Franks et al., Nature* 396: 324, 1998; *Jevtovic-Todorovic et al., Nature Med.* 4: 460, 199; *Yamakura and Harris, Anesthesiology, 20008*).

30 Based on these observations, experiments were carried out in the context of the present invention, with the aim of determining the neuroprotective effects of N₂O and of xenon, on neuronal death induced by transient 35 cerebral ischaemia ischemia in rats.

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In order to demonstrate the beneficial effect of administering N₂O or xenon on brain cells subsequent to cerebral ischaemia ischemia, adult Sprague-Dawley rats weighing 350 g were subjected to the following
5 experimental protocol.

On day 1, focal ischaemia ischemia was induced in each of the rats by middle cerebral artery occlusion (MCAO), for a period of 1 h 30 minutes.

10 The transient focal cerebral ischaemia ischemia by MCAO is obtained conventionally by introducing a flexible nylon thread 1, represented diagrammatically in Figure 1 (length 6.5 mm, diameter 180 µm), a portion 2
15 of the proximal end of which has a diameter greater than that of the thread (length 3 mm, diameter 380 µm), into the vascular system of the rat, as far as the region of the ipsilateral hemisphere so as to cause an embolism therein, i.e. an ischaemia ischemia.

20 Next, the rats are reperfused for 10 to 20 minutes, and are then made to inhale several mixtures of gases, namely:

25 - mixture No. 1: air (control)
 - mixture No. 2: N₂O (75% vol), the remainder being oxygen (25%)
 - mixture No. 3: xenon (50% vol), the remainder being oxygen (20 to 25%) and nitrogen (30 to 25%),
30 respectively
 - mixture No. 4: xenon (75% vol), the remainder being oxygen (25%).

On day 2, i.e. 24 hours after reperfusion, the rats are
35 killed, the brains are recovered and frozen, and thin

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sections 40 µm thick are cut and then stained with cresyl violet, as shown in Figure 5.

5 The volume of neuronal death is calculated, from the sections obtained after staining, in a conventional manner using an appropriate, commercially available conventional program.

10 In fact, as shown diagrammatically in Figure 2, the cerebral ischaemia ischemia engenders, in general, in 24 hours, an infarction in the region which has been subjected to ischaemia ischemia (penumbra), leading to neuronal death in the brain cells present in a considerable portion of this region.

15 The results obtained during these measurements have been recorded in Figures 3a to 3d, which make it possible to visualize the post-cerebral ischaemia ischemia neuroprotective effect of mixtures No. 2 to 4 above, in comparison with mixture No. 1 (air) which serves as a control.

25 Thus, Figure 3a clearly shows that inhalation by the rats of nitrous oxide (N_2O) or of xenon (Xe) subsequent to an ischaemia ischemia makes it possible to considerably reduce the total volume of infarction, since a decrease in this volume of approximately 50% can be achieved in the case of inhalation of mixtures No. 2 and No. 3 instead of air (mixture No. 1 acting as 30 control), and of approximately 30% when mixture No. 4 is inhaled.

In this respect, it will also be noted that inhalation of 35 50% by volume of xenon (mixture No. 3) is more effective than inhalation of a higher dose of xenon, namely 75% (mixture No. 4), which implies that the most

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effective dose appears to be closer to 50% than to 75% with regard to xenon.

Figures 3b to 3d confirm the results of Figure 3a,
5 since they make it possible to observe that inhalation of xenon or of N₂O makes it possible to decrease, respectively, the post-ischaemic ischemic volume of cortical infarction (Fig. 3b), the post-ischaemic ischemic volume of striatal infarction (Fig. 3c) and
10 the post-ischaemic ischemic volume of oedema (Fig. 3d), compared to inhalation of air (control = mixture No. 1).

Based on this observation, complementary examinations
15 were carried out in order to determine the neurotoxic effects of the xenon and of the nitrous oxide (N₂O), at various amounts, compared to air, on brain receptors of the NMDA type.

20 The results of these examinations are reported in Figure 4, which clearly shows that the administration of xenon or of nitrous oxide engenders a smaller volume (in mm³) of deteriorated NMDA receptors than the control (air), this being with the nitrous oxide given
25 at a dose of 50% or 75% by volume (remainder = 25% of O₂) and the xenon given at a dose of 50% or 75% (remainder = mixture of 25% of O₂ + 25% of N₂, or, respectively, 25% of O₂).

30 However, a neurotoxic effect which is variable according to the dose administered thus emerges, leading to the observations that N₂O at 75% and xenon at 50% by volume are more neuroprotective than N₂O at a dose of 50% and xenon at a dose of 75%.

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In other words, these data confirm that administration by inhalation of N₂O at a dose of 75% by volume (or less) or of xenon at a dose of 50% by volume (or less) engenders a neuroprotective action with respect to 5 cerebral ischaemia ischemia and other similar excitotoxic diseases.

The inhalable medicinal product according to the invention is packaged in pressurized gas containers, 10 such as gas bottles, and is dispensed to the patient via an appropriate system for administering gas, equipped with a breathing mask, a tracheal catheter, or the like.

15 Consequently, in the context of the invention, the use of gaseous nitrous oxide (N₂O) will be preferred to that of xenon, for producing a medicinal product intended to treat, minimize or prevent post-ischaemic ischemic brain cell deteriorations, such as strokes.